

Abstract No. Fede0144

**Following Pericellular Activity of Matrix Metalloproteinases by Infrared Micro-spectroscopy**

Silvina Federman (Weizmann Inst.), Arnon Grafit (Weizmann Inst.), Lisa M. Miller (NSLS, BNL) and Irit Sagi (Weizmann Inst.)

Beamline(s): U10B

**Introduction:** The extracellular matrix (ECM) is a macromolecular three-dimensional structure that controls the organization of tissues. One of its main components is collagen. Proteolytic cleavage of ECM macromolecules by Matrix Metalloproteinases (MMPs), a family of cell-secreted enzymes, is involved in normal physiological processes, such as embryonic development, and wound healing, as well as in pathological conditions, such as chronic inflammation, and tumor invasion [1]. There is insufficient knowledge on the localization and the mode of ECM degradation around the cell. To provide new insights to these questions we utilized infrared micro-spectroscopy to follow proteolytic processes, induced by invasive cancer cells, on insoluble collagen-based ECM models (matrigel).

**Methods and Materials:** Highly invasive fibrosarcoma cells were incubated on matrigel matrices for 24 hours in the presence or the absence of a broad-range MMP inhibitor. Infrared micro-spectra of the matrix were collected near and far away from the cell. Chemical images were generated by correlation analysis, in order to detect areas of non-degraded and degraded collagen around the cell. Cells were detected by integration over the 1200-1300  $\text{cm}^{-1}$  band (phosphate groups of nucleic acids).

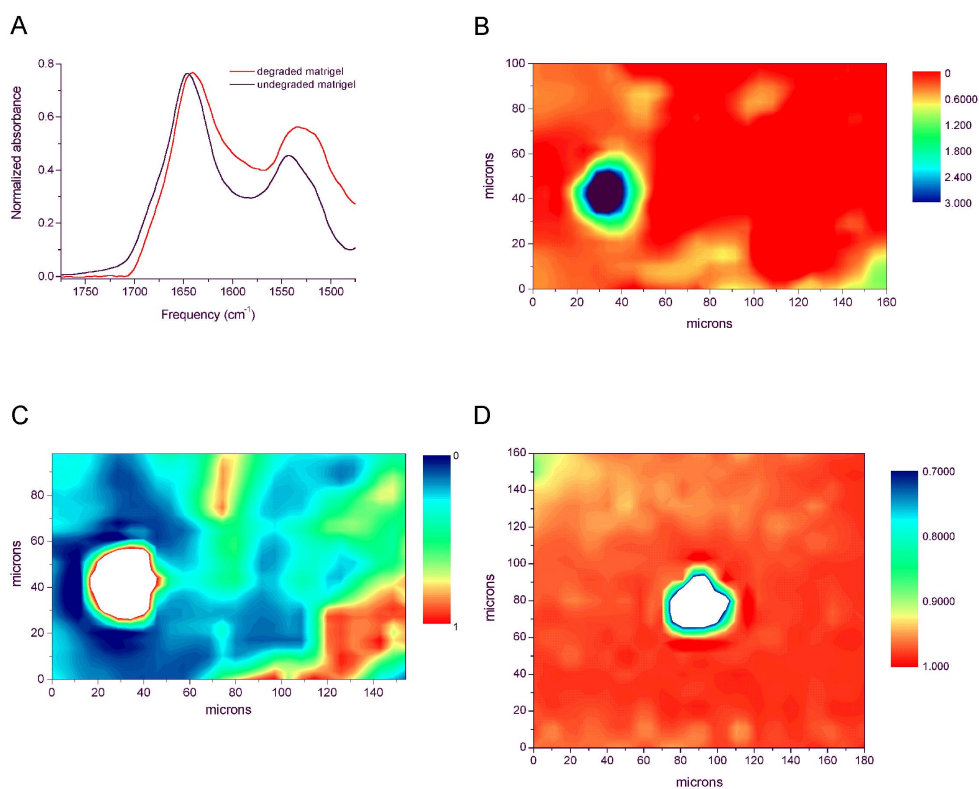
**Results:** Upon degradation of collagen by MMPs there is a shift in the absorption frequency of the amide I transition of collagen from 1652 $\text{cm}^{-1}$  to 1647 $\text{cm}^{-1}$  (figure 1A). Concomitantly, there is an increase in the absorption intensity of the amide II transition. The shift in the frequency of the amide I transition indicates that proteolysis by MMPs induces unwinding of the collagen triple-helices into single  $\alpha$ -chains [2]. Chemical image in figure 1B shows the localization of the cell on the matrix. The image shown in figure 1C reveals degradation patterns of cell-produced MMPs, which are not detected in cell samples incubated in the presence of inhibitor (figure 1D).

**Conclusions:** The detected degradation patterns suggest that proteolytic events by cell-produced MMPs are focalized in specific areas around the cell boundary. A front of intense degradation, followed by milder degradation in areas far away from the cell, is detected on one side of the cell only. Degradation by cell-produced MMPs is performed by the joint action of diffusion and unwinding of the collagen macromolecules within the physiological matrix.

**Acknowledgments:** This study was supported by the Maurizio and Clotilde Potecorvo Foundation

**References:** [1] H. Nagase and J.F. Woessner, "Matrix Metalloproteinases," *Journal of Biological Chemistry* **274**, 21491-21494.

[2] S. Federman, L.M. Miller and I. Sagi, "Following Matrix Metalloproteinases Activity Near the Cell Boundary by Infrared Micro-spectroscopy," *Matrix Biology*, **in press**



**Figure 1** Detection of pericellular proteolysis on collagen-based ECM models (matrigel). (A) FTIR spectra of matrigel, measured far from the cell (black), and near the cell boundaries (red). (B) IR image of the cell on the matrix, generated by integration over the 1200-1300  $\text{cm}^{-1}$  band. The area in black surrounded by a green boundary represents the cell. The red areas around the cell represent the matrix where no phosphate spectral signature could be detected. (C) IR image generated by correlation analysis. Each second derivative spectrum of matrigel, near and far from the cell, was correlated to the second derivative spectrum of undegraded matrigel. Blue indicates degraded matrix, red indicates no degradation of the matrix. The cell is encoded in white. (D) IR image (generated as in C) of a cell on the matrix incubated in the presence of MMP inhibitor, shows no degradation patterns on the matrix.